

Analyses of BioVOCs variation related to vegetation predominance in the Natural Park of Ampezzo Dolomites, UNESCO world heritage area of Dolomites

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Abstract: The determination of emitted Biogenic Volatile Compounds (BioVOCs) profile via Field Air Analysis (FAA) – Solid Phase Micro Extraction (75 μ m Carboxen/PDMS portable field sampler) was performed using GC-MS. The six sampling sites located in the Natural Park of Ampezzo Dolomites, part of the UNESCO's World Heritage List, are characterized by different predominating vegetation. The ubiquitous compound hexanal was chosen as internal standard, in order to compare the amounts of the different compound in the different sites. The main terpenes identified were α -pinene, β -pinene, δ -3-carene and d -limonene, while 1,8 cineole was the highest abundant oxygenated terpene. In the site next the national road a higher amount of benzene derivatives (benzene, toluene, *o*-xylene, *p*-xylene and ethylbenzene) were observed. In the site relatively close to the road a marked decrease of benzene derivatives was observed. In the sites close to conifers (*i.e.* Fir, Pine and Larch) the terpenic content observed was higher than in the site close to the mixed forest (mainly large-leaf and some conifers). Some terpenes were observed also in the high-quote meadow site, although of lower abundance than in the other sites. The highest abundance of terpenes was observed in the *Pinus cembra* (Swiss Pine) site.

Keywords: Solid phase microExtraction; organic volatile compound; Terpene; plant emission; mass spectrometry; gas chromatography.

Abbreviations:

BioVOCs--Biogenic Volatile Organic Compounds; EO--Essential Oil; SPME--Solid Phase MicroExtraction; PFS--Portable Field Sampler; HS--

Head Space; FAA--Field Air Analysis; GC--Gas Chromatography; *r.t.*--retention time; MS--Mass Spectrometry; TIC--Total Ion Current; LLE--Liquid Liquid Extraction; SFE--Supercritical Fluid Extraction

Introduction

During their life, plants produce a wide variety of organic volatile compounds such as alkanes, alkenes, alcohols, esters, carbonyls and terpenes (hydrocarbon compounds based on the repeating unit of isoprene, C_5H_8). These set of compounds are usually called Biogenic Volatile Organic Compounds (BioVOCs) (Bursker 1940; Isidorov 1985; Helming 1989; Nakajima 1989). BioVOCs are produced by vegetation as secondary metabolites and they appear to be a defense against pathogens, herbivores and seem implicated in wound healing. They could be linked to thermo-tolerance, help plants in photosynthesis at higher temperatures, avoiding the damage of the cellular membrane and limiting the oxidative stress. Recent studies report that BioVOCs can attract pollinators and can mediate the communication between the plant and other organisms (Llusia 2002). BioVOCs have reported antibacterial and antifungal properties. They are able to influence (and to be influenced by) a great number of ecological processes in soils, including inhibition of methane oxidation, nitrification, saprotrophic fungal activity and inhibition or stimulation of mycorrhizal colonization (Robinson 2009).

Terpenes are the main volatile constituents of plant emissions, plant resins and plant distillation products (essential oils, EOs) (Clayden 2001). People have used these perfumed oils for thousand years, for their aromas, for their bacteriostatic and antiseptic properties, in religious rituals and in the early medicine (Guentert 2007). Several investigations about the biotic and abiotic factor that influence the terpenes production have been performed. In particular the air and soil temperature, rainfall and relative humidity effect on terpenes production have been investigated by Dorn (2005), (using Radiello sampler to collect terpenes emitted from plants) showing that in drought condition BioVOCs production is enhanced in apple tree site. Similar results have been

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obtained by Blanch (2009) in *Quercus ilex* (Holm Oak) and *Pinus halepensis* (Aleppo Pine) greenhouse, extracting terpenes via Liquid-Liquid Extraction (LLE) from leaves; they found that drought significantly increase the total terpene contents. Other relevant biotic and abiotic factors are plant competitions (Fernandez 2006), soils mineral contents (calcareous soils or siliceous soil) (Ormeno 2008), and pollution by antropogenic sources (Supuka 1997).

Terpenes can affect human health. Several studies have been accomplished regarding to the antibacterial and antimicrobial properties of EOs and some of their major constituents (Burt 2004; Averbek 2008; Guentert 2007). The antimicrobial properties of EOs are attributable to monoterpenes, sesquiterpenes and related alcohols, other hydrocarbons and phenols (Zygadlo 2000). Oxygenated monoterpenes such as 1,8-cineol and alcohols such as linalool were reported to be active against several species of bacteria (Tzakou 2001; Senatore 2005; Zou 2004; Demirci 2002; Sonoboli 2005; Delaquis 2002). Monoterpenes (*i.e.* sabinene, terpinene, limonene, α -pinene and β -pinene) and sesquiterpenes (*i.e.* farnesol and β -phellandrene) have shown strong specific antimicrobial properties, and antifungal activity (Koroch 2007). Some studies on EOs activity indicate that some their minor components are critical for antimicrobial and antifungal activity, probably due to a synergic effect among the major and the minor constituents (Burt 2004; Mastelic 2005), even if some antagonistic effects have been observed (Cox 2001). Terpenoids are lipophilic agents and act disrupting the cell membrane integrity and permeability (Ultee 2000). Antiviral activity against *Herpes simplex virus* type 1 and type 2 of *Eucalyptus globulus* (Blue gum) or *Mentha piperita* (Peppermint) EOs has been described (Schnitzler 2001; Schnitzler 2003). Anticholinesterase activity and antinociceptive effect was reported for 1,8-cineole (Santos and Rao 2000), while antiulcer activity was showed by linalool (Barocelli 2004). Phenolic monoterpenes (*e.g.* thymol) seem to be the highly active in muscarinic receptor blocking calcium antagonist (Koroch 2007).

Several experimental and population-based studies suggest that monoterpenes in diet play an important role in the cancers prevention (Greenwald 2001; Schwartzmann 2001; Crowell 1999; Elson and Yu 1995; Gyllenhaal 2004). Perillyl alcohol and *d*-limonene are of great clinical interest. In particular *d*-limonene exhibited chemo-preventive and therapeutic effects against chemically-induced mammary tumors in rats (Phillips 1995; Kaji 2001; Gould 1989) and against metastasis of human gastric cancer (Lu 2004).

Phenols play an important role as antioxidant agent present in EOs. Reactive oxygen species (ROS), in particular hydrogen peroxide, may be the causative factor involved in many human degenerative diseases. Phenols possess some preventive and therapeutic effects in this kind of diseases (Koroch 2007).

All these researches put in evidence the role of vegetation and derived products in human health. Although terpenes concentration in air is some orders of magnitude lower than that present in EOs (about 10–500 $\mu\text{g}/\text{m}^3$ against some % v/v in EOs), their effect can be relevant for human health. It is to remark that a great number of sanatoriums (in the late 19th century and until the

middle 20th century) for tuberculosis and other respiratory diseases were present in the Dolomites area (now in the UNESCO's World Heritage List), mainly built in coniferous-rich zones, far away from the relatively crowded towns.

Several studies have been accomplished in order to determine either the qualitative or the quantitative composition of BioVOCs emitted (using Head Space, HS, or Field Air Analysis, FAA) or produced (using extraction techniques) by plants. HS and FAA sampling are non-destructive methods for collecting volatiles, giving a more realistic picture of the BioVOCs emitted. Theoretical bases and various applications of SPME have been presented by Pawliszyn (1997) as well as in numerous publications (Zhang and Pawliszyn 1993; Steffen and Pawliszyn 1996; Pawliszyn 1999). Isidorov (1985) have performed various measurements, using different sampling methods (sampling tubes, sampling bags, and SPME) and analyzing a wide range of plants and canopy of coniferous forest. They put in evidence that the amounts of terpenes quantified in *Pinus sylvestris* (Scots Pine) is strongly influenced by the sampling method; strong differences were found comparing the different HS and FAA techniques and HS or FAA with LLE results.

Some explanation arises by the researches of Juttner (1988) sampling air of a *Picea abies* (Norway Spruce) rich forest where bornyl-acetate, an important constituent of the needles of *Picea abies*, was found only in trace concentrations. Comparing the different terpene concentrations in the air with those in the needles of *Picea abies*, their different volatilities cannot be the only explanation. They emphasized the presence of degradation processes in the air, controlled by O_3^* , OH^* and NO_3^* radicals. Thus, high rate constants for the reactions of ozone and OH^* with monoterpenes can provoke their rapid elimination after emission into the air. Fernandez (2006) pointed out the differences in permeability of terpenic compounds to cell membranes. Moreover, considering the differences in plant structures, some compounds can be directly released by some classes of plants while other classes can store terpenes in specialized pools prior their emission. If FAA methods are effective to measure the flow of emitted terpenes, extraction methods can gives accounts for the overall terpenic content, with any regard to their volatilities.

Despite the high influence of external factors in BioVOCs productions and the influences of the analysis method, some general trend can be described. Terpenes emission is significantly higher during the summer rather than in winter, with terpenes concentration of about 1000 ng/m^3 and 50 ng/m^3 respectively (Juttner 1988; Hakola 2003; Lappalainen 2009). Terpenes emission shows a diurnal variation, reaching the maximum concentration during the daylight of about 600 ng/m^3 (for α -pinene), against the 150 ng/m^3 for the same molecule during the night (Bufler and Wegmann 1990; Holzke 2006).

Generally the terpenic content of the arboreous species is quite similar. Nevertheless some plants emits characteristics terpenes, *e.g.* *Picea abies* is found to emit significant amounts 1,8-cineole (Juttner 1988; Isidorov 2003; Hakola 2003) and camphor, with trace of bornyl acetate (Juttner 1988). Deciduous trees *i.e.* *Fagus sylvatica* (Common beech) or *Betula pendula* (Silver Birch) are characterized by the strong emission of sabinene (Hakola 1998;

Holzke 2006). Thus by considering the particular emitted terpene it could be possible to recognize the dominant vegetation in the sampling site. As observed by Isidorov (1985) the way of sampling strongly affect the results in terms of total amount of detected terpene. In this way while measurement via SPME or Radello™ or Tenax™ (see Table 1 for references) are relative to the terpenes emission, the solvent extraction-based methods (LLE or Supercritical Fluid Extraction, SFE) are related to the overall terpene production (Marriott 2001; Pourmortazavi and Hajimirsadeghi 2007).

To our knowledge, the BioVOCs of only few plant species of the Dolomite's area have been extensively investigated and any in-field terpene determination has not been carried out. For this reason we undertook the present study, having the aim of evaluating the BioVOCs variation related to vegetation predominance in the Natural Park of Ampezzo Dolomites, in order to give an evaluation of the healthiness of this natural environment.

Materials and methods

Field site and measurement period

Investigation on BioVOCs emitted by plants was carried out in natural conditions. The sampling site was Lerosa forest, in the Natural Park of Ampezzo Dolomites the 29th of July 2009, a sunny and brightly windy day. The site is located about 15 km from the centre of Cortina d'Ampezzo town (46°37'N 12°10'E, Belluno, Italy). Six different sampling sites were chosen as reported in Fig. 1. The first sample (A) was kept in a wood (com-

posed by large-leaf trees and conifers), near the national road 51 "Alemagna" (elevation 1,400 m), the second sample (B) was collected in a wood mainly composed by Fir species (elevation 1,600 m); the third sample (C) was collected in a wood composed by Larch and Mugo species (elevation 1,940 m); the fourth sample (D) was collected in a high-quote meadow (elevation 1970 m); the fifth (E) was collected in a *Pinus mugo* (Mugo pine) rich wood (elevation 1,970 m) and the sixth (F) one was collected in a *Pinus cembra* dominating wood (elevation 1,970 m).

Experimental

Standards

Benzene, *o*-ethyltoluene and phenol were purchased from Carlo Erba Reagenti (Milano, Italy), toluene was purchased from J. T. Baker (Deventer, Holland) while linealool, α -pinene and β -pinene were purchased from Aldrich (Buchs, Switzerland).

SPME fiber

Several kinds of fibers are commercially available in the analysis of volatile compounds. According to our experience based on previous experimental work (Traldi 2008), supplier recommendations (Supelco, Bellefonte, PA, USA) and literature (Isidorov 2003), the SPME Portable Field Sampler (PFS) fiber coated with 75 μ m of Carboxen/Polydimethylsiloxane stationary phase 504831 (CAR/PDMS) (Supelco, Bellefonte, PA, USA) was considered to be the most suitable for the molecule of interest (terpenes and oxygenated terpenes). Fibers were conditioned following the supplier specification (280°C for 0.5 hour).

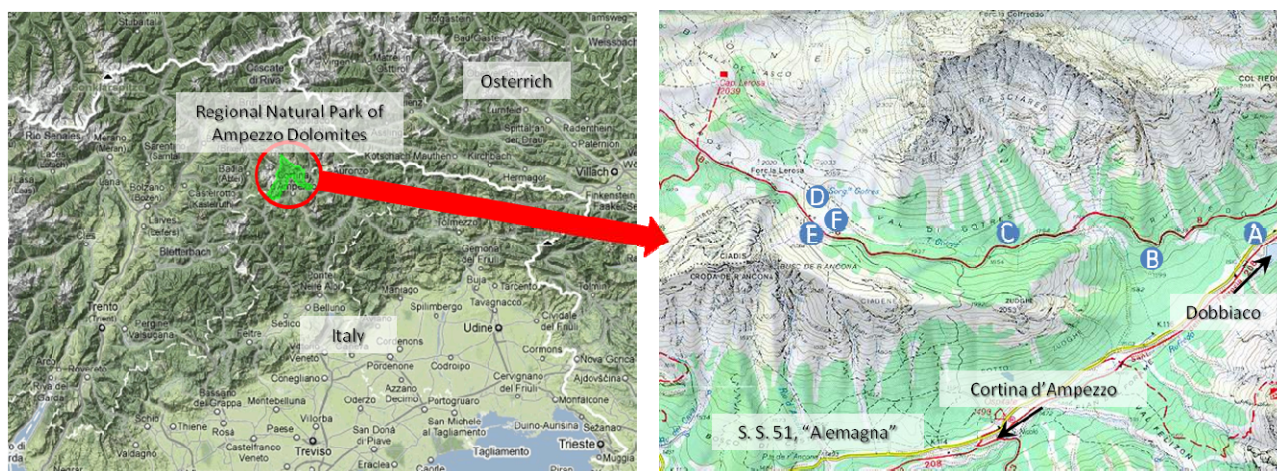


Fig. 1 Location of the Natural Park of Ampezzo Dolomites and locations of the sampling sites. Sites are noted with A, B, C, D, E and, F.

Sample conservation

Once the samples were collected (5 h of fiber exposition) the PFSs were stored in a sealed aluminum bag and kept at 4°C until the analysis, performed one day after the sampling campaign.

GC-MS analysis

FAA-SPME analysis was performed using an Agilent 6850 gas chromatograph coupled to an Agilent 5975C quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) op-

erating in EI conditions (70 eV, 35 mA). The samples were introduced via GC, utilizing a HP-5 column (30 m, 0.250 mm ID, 0.25 μ m film; Agilent Technologies, Santa Clara, CA, USA). The injection port temperature was set at 250°C. The GC was operating in splitless mode for 10 s and hence in split mode (10:1). Helium (AirLiquide Italia Service, Milano, Italy) was the carrier gas, kept at constant flow of 1.0 mL/min. The oven temperature was set at 40°C for 5 min, then at 4°C/min to 150°C and at 15°C/min until 300°C. The quadrupole mass analyzer operated in scan

mode from m/z 40 to m/z 800. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions, by comparison of EI induced fragmentation patterns with those of pure compounds and by comparison with mass spectra library data (National Institute of Standard and Technology, NIST, MS 05 spectral database).

Results and Discussion

As described in the introduction section, the BioVOCs emission strongly depends on a wide number of variables, such as temperature, humidity, drought, soil and other biotic and abiotic factors. Hence comparison between literature data, even for the same species, shows a wide spread of results, sometimes different from the qualitative but mainly from the quantitative point of view. Moreover, the sampling methods can affect the results of the analysis so that some compounds in some studies were undetectable while others (using GC separation) may coelute. These

points were critically considered in the present investigation.

We focused our attention to the plants which represent the largest population in the monitored sites and consequently compared our results with the literature data describing *Picea exelsa* (Norway Spruce), *Picea abies*, *Pinus abies* (Dormont 1998; Jansson 1999; Isidorov 2003; Robinson 2009), *Abies alba* (Silver fir), *Abies pectinata* (Silver Fir) (Dormont 1998; Simon 2006), *Pinus cembra* (Dormont 1998), *Pinus mugo* sub. *Uncinata* (Dormont 1998), *Pinus mugo* sub. *Turra* (Bambagiotti and Vincieri 1972; Garneau 2005), *Betula pendula* (Hakola 1998; Holopainen 2005), *Larix decidua* (European Larch) Miller and, *Fagus sylvatica* (Holzke 2006; Dewulf 2010). It is worth of note that in the studies just listed different extraction methods were used as summarized in Table 1. Except Isidorov (2003), all the other authors focused their attention on some of the main components of the whole terpenic content of plant emissions. In these studies it is generally recognized that sabinene is mainly emitted by deciduous plants and 1,8-cineole is mainly due to *Picea abies*. Nevertheless Simon (2006) found consistent amounts of 1,8-cineole in *Abies alba*.

Table 1. Evaluation of the relative abundances of selected terpenes from literature

Authors	plant species	Sampling method	Adsorbing material	Organic Compound										
				tricyclene	α -pinene	camphene	phellandrene	β -pinene	β -myrcene	3-carene	o/m-cymene	d-limonene	1,8-cineole	camphor
Isidorov 2003	<i>Picea</i> <i>Abies</i>	HS-SPME GCMS	CAR/PDMS	-	21.9	6.3	-	2.5	2.5	-	1.9	3.1	61.9	-
Dormont 1998	<i>Picea Abies</i> (<i>foliage</i>)	HS-GC bag	Amberlite XAD2	traces	18.5	6.9	with limonene	29.1	20.3	11	-	14.2	-	-
Jansson 1999	<i>Picea abies</i>	HS-GC	Tenax TA	-	29.1	6	-	17.9	20.5	12	-	14.5	-	-
Juttner 1988	<i>Picea abies</i>	HS-GC	Tenax TA	3.7	22.3	29.7	-	30.3	with carene	7.4	-	-	-	6.5
Robinson 2009	<i>Picea abies</i>	LLE	n-hexane	-	23.3	3.5	-	58.1	8.1	2.3	-	4.7	-	-
Gerneau 2005	<i>Pinus Mugo</i> <i>Turra</i>	EO		0.7	30	2.9	0.3	13.1	4.5	40.1	1.8	6.5	-	-
Dormont 1998	<i>Pinus Mugo</i> <i>Uncinata</i>	HS-GC bag	Amberlite XAD2	-	29.1	2.7	with limonene	16.5	28.8	-	-	23	-	-
Dormont 1998	<i>Pinus Cembra</i>	HS-GC bag	Amberlite XAD2	-	33.6	0.6	with limonene	15.8	2.7	-	-	47.3	-	-
Simon 2006	<i>Abies Alba</i>	HS-GC	Amberlite XAD2	-	13	17.4	with limonene	10.3	18	-	-	32.6	8.7	-
Dormont 1998	<i>Abies Alba</i> (<i>foliage</i>)	HS-GC bag	Amberlite XAD2	traces	52.5	0.9	with limonene	12.3	1.9	-	-	32.3	-	-
Hewitt 1997	<i>Pinus Pinea</i>	GC-MS	Tenax TA	-	11.3	-	-	4.4	8.9	-	-	55.2	20.2	-
Hakola 1998	<i>Populus Tremula</i>	HS-GC	Tenax TA	-	24.5	1.6	-	8.8	-	34.3	-	30.8	-	-
Hakola 1998	<i>Betula Pendula</i>	HS-GC	Tenax TA	-	40.9	22.4	-	11.8	-	21.1	-	3.7	-	-
Holopainen 2005	<i>Betula Pendula</i>	HS-GCMS	Tenax TA	-	0.9	-	-	with mircene	with b-pinene	-	96.8	1.4	0.9	-
Holzke 2006	<i>Fagus Sylvatica</i>	HS-GC	Tenax TA/Carbotrap	49.5	3.7	-	13.2	7.4	12.1	5.8	-	8.4	-	-
Dewulf 2010	<i>Fagus sylvatica</i>	HS-GC	Tenax TA/Carbotrap	-	29.5	-	21.2	20.7	-	-	-	28.5	-	-

Our analysis campaign has been performed in six different sites in the Natural Park of Ampezzo Dolomites (Fig. 1), part of the UNESCO's world heritage list. Each sampling site is charac-

terized by different dominating vegetation. In particular the first sample (A) was collected in a wood (composed by large-leaf trees and conifers), near the national road 51 "Alemagna", quite

highly crowded by cars, 1,400 m above the sea level. The second sample (B) was drawn in a wood mainly composed by Fir species, 200 m higher than the street. The third sample (C) was collected in a wood composed by Larch and Mugo species, at 1,940 m above the sea level and far away (5 km) from the street. The fourth sample (D) was collected in a high-quote meadow (1970 m above the sea level), quite close to sampling sites E and F. The fifth (E) was collected in a *Pinus mugo* rich wood and the sixth one (F) was collected in a *Pinus cembra* dominating wood. In order to evaluate possible contamination of the SPME fibers used for sampling, in the storage bag (kept at 4°C) a blank fiber, carried around the sampling sites but not exposed at the vegetation emissions has been analyzed. The samples were collected during the same brightly windy and sunny day (29th July 2009), exposing the fibers for the same time (5 hours, from 10 am to 15 pm).

Although SPME can be used for both qualitative and quantitative analysis our data can be considered only semi-quantitative.

The areas of the detected peaks were compared with the area of hexanal, an ubiquitous and available at almost the same concentration analyte (Nakajima 1989; Ciccioli 1993, both indicating hexanal concentration in the range 50–250 ng/m³), thus obtaining the relative concentrations of the different compounds detected in the six different sampling sites (see Table 2 for detailed data).

In each chromatogram, obtained from the six sampling sites, three main zones (Fig. 2, Table 2 and Fig. 3) can be distinguished. In the first zone (corresponding to retention times, *r.t.*, from 2.50 to 11.20 min) the aromatic hydrocarbons like benzene, toluene, ethylbenzene and, xylenes or aldehydic compounds predominate. They are due to anthropogenic sources or can be considered ubiquitous compounds, in particular hexanal and heptanal (Nakajima 1989; Ciccioli 1993). The second zone (*r.t.* between 11.20 to 15.80 min) is dominated by monoterpene hydrocarbons. In this zone the most abundant peaks are usually due to α -pinene, β -pinene, δ -3-carene and, *d*-limonene (see Table 2).

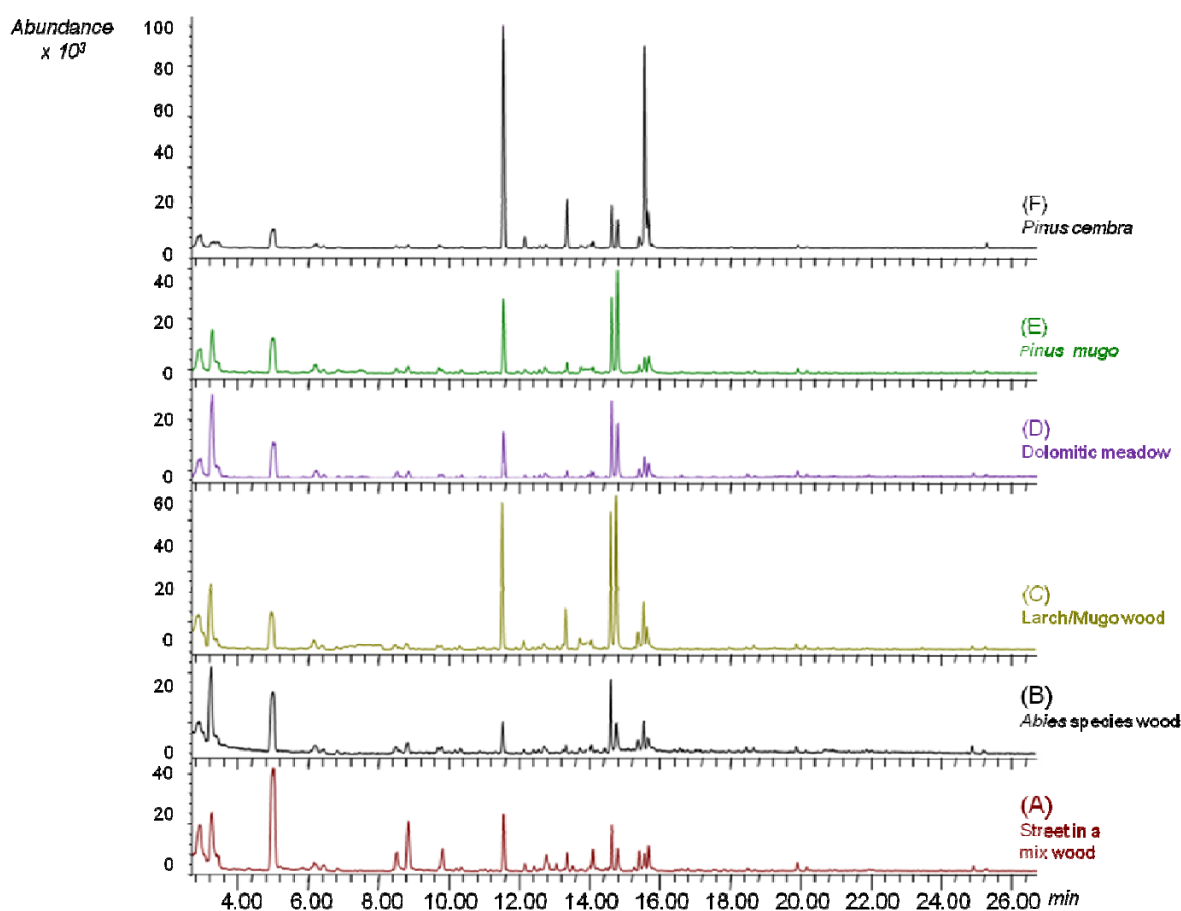


Fig. 2. Merged chromatograms of the six sampling sites.

The third zone (*r.t.* between 15.80 to 26.00 min) is dominated by oxygenated monoterpenes (C₁₀H₁₆O or C₁₀H₁₈O). In this case the main peaks are due to 1,8-cineole, β -pinone and bornylacetate.

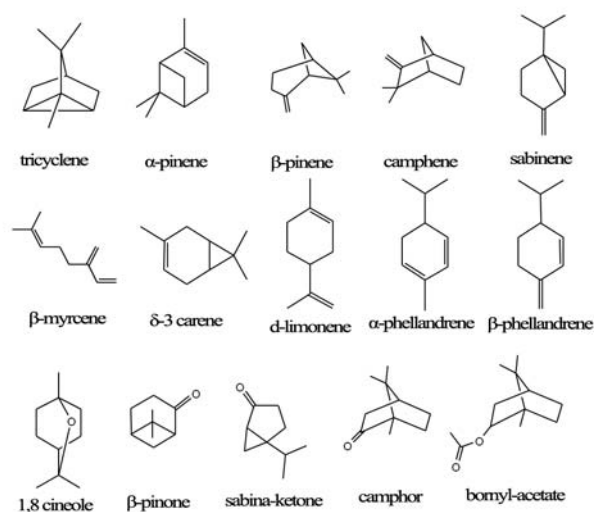
Comparing the chromatograms obtained in different sites (Ta-

ble 2) we can notice some significant differences. The structure of some of the recognized terpenes and related compounds are reported in Fig. 3 and the histograms of Figs. 4–6 summarize the obtained results, reported in detail in Table 2.

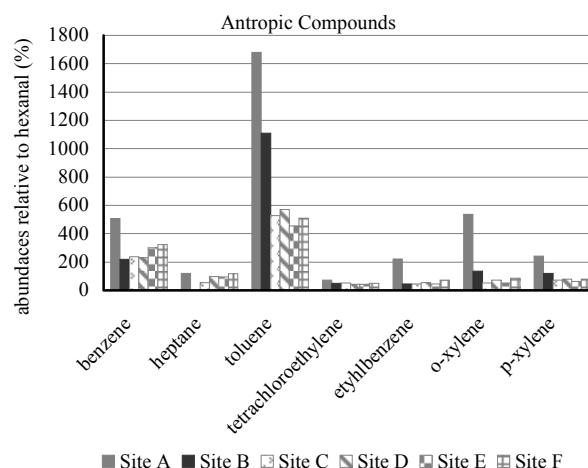
Table 2. Retention times and abundances relative to hexanal (%) of the compounds recognized in FAA-SPME analysis via GC-MS

	r.t. (min)	Site A	Site B	Site C	Site D	Site E	Site F
benzene	2.90	510	220	240	230	300	320
heptane	3.40	120	-	60	100	90	120
toluene	5.00	1680	1110	530	570	450	510
hexanal	6.20	100	100	100	100	100	100
tetrachloroethylene	6.45	70	50	50	40	40	50
ethylbenzene	8.50	220	50	50	50	50	70
o-xylene	8.85	540	140	50	70	50	90
p-xylene	9.80	240	120	70	80	60	80
nonane	10.20	30	30	20	-	10	-
heptanal	10.35	40	40	30	30	20	40
tricyclene	11.00	20	-	30	-	20	50
α -pinene	11.55	220	230	870	320	400	2430
camphene	12.15	70	50	60	30	30	120
isocumene	12.40	50	40	20	20	10	20
2 ethyl-hexanal	12.60	-	50	20	20	20	20
benzaldehyde	12.75	190	90	40	50	60	70
triethylbenzene	13.05	50	-	-	-	-	-
salinen/phellandrene	13.25	-	30	30	-	10	30
β -pinene	13.35	140	-	220	50	60	490
cumene	13.50	50	30	-	-	-	-
phenol	13.75	-	40	80	-	40	30
β -myrcene	14.10	140	60	50	40	30	80
δ -3 carene	14.80	190	230	810	350	480	310
o/m cymene	15.40	140	70	90	60	40	150
d-limonene	15.55	120	210	270	140	90	2000
eucalyptol	15.65	150	110	160	110	120	410
p-dimethyl styrene	18.00	-	30	20	-	-	40
β -pinone	19.90	60	40	30	40	30	40
camphor	20.15	50	20	30	20	20	30
sabina-ketone	20.50	-	20	10	-	10	-
pinanediol	24.90	50	60	30	30	10	10
bomylacetate	25.30	20	20	10	10	10	60

Site A: Street in a mix wood; Site B: Abies species wood; Site C: Larch/Mugo wood; Site D: Dolomitic meadow; Site E: Pinus mugo; Site F: Pinus cembra.

**Fig. 3** Structures of monoterpenes and related compounds recognized via FAA-SPME analysis in plants

As expected, the anthropogenic hydrocarbons abundance is higher near the national road 51 “Alemagna” (Site A) while it is lower in high altitude sites (Fig. 2 and Table 2). While benzene abundance decreases around 20%–50% passing from the site A to the other ones, the decrease in abundance of toluene, ethylbenzene and xylenes is higher than 70% (Fig. 4). The most of Total Ion Current (TIC) in chromatogram A in Fig. 2 is due to the aromatic species. The TIC due to terpenes and related compound is of low intensity. The overall terpenic content in site A is comparable with that of the site B (even if in the first case there are some large-leaf trees), but significant lower than those of the other sites (see Fig. 5). This could be explained considering the lack of competitive adsorbed species (lower amounts of terpenic species which compete with aromatic compounds partitioning equilibria). Some experiments have shown that the presence of NO^* , O_3^* and light ($h\nu$) leads to severe degradation of some monoterpenes, depending on their structure. In particular α/β pinene and d -limonene are readily degraded in air (Juttner 1988); as known, NO^* and O_3^* can be generated directly and indirectly by fossil-fuel motors and hence it is not surprising their lower concentration in the polluted or highly UV-ray exposed sites (A and B, nearest the road).

**Fig. 4** Anthropic contribution at the overall total ion current, normalized by heptanal area. Site A: Street in a mix wood; Site B: Abies species wood; Site C: Larch/Mugo wood; Site D: Dolomitic meadow; Site E: Pinus mugo; Site F: Pinus cembra.

Sabinene, expected in large amounts in deciduous-tree dominating forest (Hakola 2003; Holzke 2006), was not found. It is to remark that sabinene was detected only in few weeks during the summer, nearby August and September in Finland. The different geographical position of Natural Park of Ampezzo Dolomites can greatly influence the emission of terpenes and sabinene in particular. Furthermore it must be emphasized that sabinene has been closely linked to the presence of *Betula pendula* trees, which is not the dominating vegetation in the Park region.

In the Fir forest site (point B in Fig. 1) the presence of higher amounts of δ -3-carene and d -limonene with respect to the other compounds can be noticed (Fig. 2B and Table 2). The presence

of α - and β -pinene and *d*-limonene can be explained either by the presence of *Fagus sylvatica* (Dewulf 2010) and, in minor extent, *Betula pendula* (Hakola 1998). The detection of δ -3-carene can be explained considering the presence of *Betula pendula* (Hakola 1998). The terpene concentration is lower than that observed in the other sites. Further compounds (trace of terpenes and oxygenated terpenes) are detected at almost the same amount than in the other zones. Also in this case the environmental pollution can be considered responsible for the low abundances of terpenes, due to their ready degradation. In fact significant amounts of anthropic compounds are found (see Fig. 5 and Table 2).

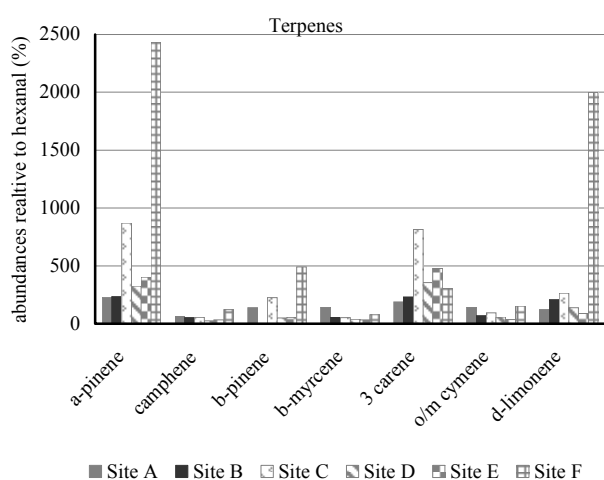


Fig. 5 Terpenic contribution at the overall total ion current, normalized by heptanal area. Site A: Street in a mix wood; Site B: Abies species wood; Site C: Larch/Mugo wood; Site D: Dolomitic meadow; Site E: *Pinus mugo*; Site F: *Pinus cembra*.

The Larch/Mugo forest site seems to emit a higher amount of terpenes; in the site C high amounts of α -pinene, β -pinene, *d*-limonene, δ -3-carene and 1,8-cineole were found. Due to the high number of different trees species in the mixed-coniferous forest (site C) it is difficult to evidence a clear relationship between dominant terpenes and plant species. Literature data indicates, accordingly to our analysis, the presence of some terpenes originating from *Picea abies* and *Abies alba*. In particular β -myrcene and camphene (Juttner 1988; Dormont 1998; Janson 1999; Moukhtar 2006; Robinson 2009) and *o/m* cymene (Isidorov 2003; Robinson 2009) are found in relevant amount. The amounts of the oxygenated terpenes is about ten times less abundant but still significant (see histogram of Figure 6). 1,8-cineole is found at remarkably abundance. It has been reported in literature only in few cases, when analyzing *Picea abies* and *Abies alba* (Isidorov 2003; Simon 2006) while camphor has been reported only in *Picea abies* (Juttner 1988). It is to remark the presence of phenol, a strongly antibacterial molecule.

The high quote meadow site (D) leads to a chromatogram showing quite weak signals. The anthropogenic compounds abundance is similar to the abundance found in the site C and

lower than that found close to the road (A and B), as reported in Fig. 4. The poor terpene contents in this site is not surprising, although it is surrounded by a wide variety of different woods, *i.e.* *Pinus mugo* sub. Turra and *Pinus cembra*. The relatively most abundant species are α -pinene and δ -3-carene, but at very low concentrations. Some terpenic species of low abundance also in the other sampling site are, for D, totally undetectable, *i.e.* tri-cyclene, cumene and salinen/phellandrene (Table 2). The situation is almost the same in the case of oxygenated compounds. Worthy of note is the total absence of phenol in site D. To our knowledge any articles reporting the terpenic contents of dolomitic-meadow zones are not available. The poor terpene content may be due to their lack of production and emission but also due to their *h v*-induced degradation processes.

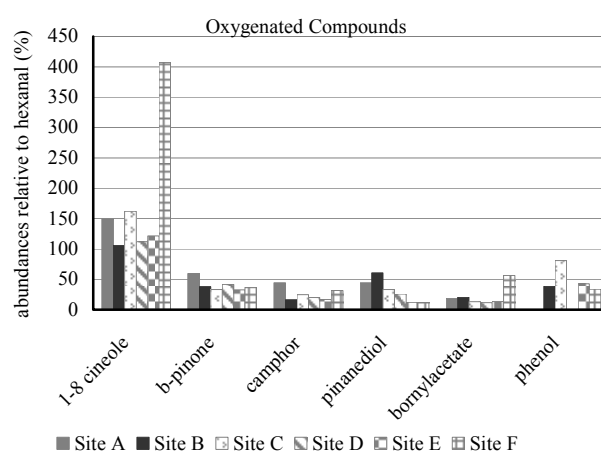


Fig. 6 Oxygen-containing molecules contribution at the overall total ion current, normalized by heptanal area. Site A: Street in a mix wood; Site B: Abies species wood; Site C: Larch/Mugo wood; Site D: Dolomitic meadow; Site E: *Pinus mugo*; Site F: *Pinus cembra*.

In *Pino mugo* sub. Turra sampling zone (E) the main peak is due to δ -3-carene (Fig. 2E and Table 2) analogously to what observed for the mixed-conifers site C. The experimental data are in agreement with those by Garneau (2005) and Bambagiotti and Vincieri (1972), obtained analyzing its EOs: high amounts of α -pinene and low amounts of β -pinene and *d*-limonene have been found also in the present investigation. The other detected compounds exhibit low abundances as described by Garneau (2005). The further molecules of interest follow the *Pino mugo* sub. Turra trend, showing low amount of other terpenes. This fact is quite surprising considering that for years Mugo-rich EO drugs have been used for breathing diseases. It is to remark that EO extraction is deeply different from SPME method with regards to the relative abundances and total amounts of terpenes. The oxygen-containing compound zone is not an exception to the overall trend that is lower amount of the analytes compared to the other sampling zones. Quite surprisingly the chromatographic outline in E is quite similar, in abundances and number of terpenes, to that of the site C.

In the *Pinus cembra* zone (F), the overall terpenic and related compound content is the highest observed in our sites (Fig. 2F and Table 2). In particular, while the anthropogenic-due compounds are practically absent (Fig. 4), the relative abundance of α -pinene, β -pinene, camphene and *d*-limonene, is higher than in the other sites (Fig. 5). These results are in agreement with those of Dormont (1998). In particular α -pinene abundance is at least three times higher than in the other sites; *d*-limonene amount is comparable to the α -pinene one and almost seven times more abundant than in the other sites. Other remarkable terpenes are δ -3-carene, cymene and tricyclene (Fig. 5). Marked differences with the other sampling sites are observed also in the case of oxygen-containing compounds. In particular it can be observed a high amount of 1,8-cineole and a significant increase in bornylacetate.

Conclusions

This work was devoted to the qualitative and semi-quantitative identification of the different emitted terpenes from six sites inside the Natural Park of Ampezzo Dolomites. Each site is characterized by different arborous species and substantial differences were found. In particular the marked decrease of aromatic compounds when leaving the street site (A) is to emphasize. Even if the sampling site B was relatively close to the site A, a marked decrease in aromatic compound has been observed. The terpenic content is higher in the sites close to conifers species (C, D and F) while it seems to be of lower intensity in the mixed forest (A). Large-leaf trees are known to emit mainly isoprene (not detected in our analysis) and low amounts of terpenes respect to conifers. The amount of terpenes in the meadow (E) is comparable with that detected in the area characterized by the presence of *Pinus mugo* and it may be due to the closeness of the two sites (Figure 1). The highest amount of terpenes was detected in the *Pinus cembra* site (F). The oxygenated compounds are of lower abundance than terpenes but still worth of note. In particular, considerable amount of 1,8-cineole has been found.

The natural progress of this work is the quantitative characterization of the emitted terpenes and the analysis of their variation during the different seasons. Another interesting challenge is the comparison between the terpenic content of the different species determined via LLE analysis and the comparison with the emitted terpenes, determined via SPME and other HS techniques such as Radiello. Terpenes and related species quantitation are of sure interest in order to evaluate the possible benefits on human health of such bioactive molecules. Dolomites can affect the settler mood for their unique skyline and breathless beauty (as recently confirmed by UNESCO) and, by the terpenes emission, also from the wellness point of view.

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